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Effect of Carbon Sources and Their Various Concentrations for Optimize in *In vitro* Micro propagation of Banana *Musa* (spp.) Basrai

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Abstract— *Banana fruit is the second vital food crop. Although the significant business crop value, the major production restrain is the accessibility of reliable and harmless material for planting. For in vitro growth of excited tissues, sources of carbon have been considered as one of the most significant factor. Types of sugars and concentrations are identified to affect the in vitro protocol success. Crop micro propagation is also facing the challenges which require to be addressed in order to improvement in its production. In this study, influence of three carbon sources such as (dextrose, sucrose and sorbitol) used to increase Murashige and Skoog medium at four applications control, 15, 30, 45 and 60g/l respectively. This study is accomplished at the laboratory of tissue culture of Plant Pathology Section, Sindh Agricutlure Research Insitute, Tandojam, Pakistan in 2017. Randomized Block Design (RBD) method with 3 replications was used for each treatment. Results indicated that sucrose give maximum result as compared to dextrose and sorbitol. Though, significant result was found in sucrose 30g/l as compared to dextrose and sorbitol concentrations.*

Keywords— ***Carbon sources, Concentrations, Media, Micro propagation, Banana Musa (spp.) Basrai.***

I. INTRODUCTION

Banana fruit crop is a significant all over the world because it gives an important income source in local and international trade (Frison et al., 1997). Banana is the general name of genus *Musa* (spp). And it has a great nutritional and profitable value in world. It is great supply of carbohydrates and proteins and supply of vitamins (Vit A, C, E, K, B1, B2, B3, B6 and B9) and minerals such as, calcium, magnesium, sodium, potassium, besides with trace amounts of carotenoids, iron and zinc (KTL, 2007). It also has been found useful against breast and colorectal cancer (Zhang, 2009; Deneo-Pellegrini et al., 1996). Banana ranked as the 2nd most vital fruit crop and it accounts approximately 22% of fresh fruit (Pua EC, 2007).

During the early nineties, in the banana fields of Sindh, Pakistan, a mysterious disease spread which occupied more than 60% area, and due to which 90% production declined. Later, the disease was recognized as banana bunchy top disease produced by banana bunchy top virus (BBTV). Virus vector is *Pentalonia nigonervosa* which contaminated plants and provide typical bunchy top emergence, which is owing to the failure of stand stiff and leaves flexibility. Due to the production heavy loss, farmers changed to other crops like cotton and sugarcane but from these crops they were not able to get high income, as compared to get from banana. So, to get the planting material which is disease free for re-cultivation of fields, all efforts were diverted, which were damaged by the bunchy top virus (BBTV) of banana. One way was to introduce the healthy germplasm from out of the country, but importing of germplasm could not adjust to the local soil or the environment. Another approach was the cleaning of existing germplasm and grows at much high rate, so that the farmer's requirements may be fulfilled (Aish Muhammad et al., 2004).

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Technology of TC provides mass propagation and clean material for planting. Production of banana under *in vitro* techniques is a greater technology over conventional method (Sucker-propagated) with respect to best yield, consistency, disease-free material for planting and true to type plants. Mass reproduction of TC (tissue culture) plants could be prepared in a short time. They are affordable to transport than conventional suckers and the coupling with virus indexing allows for protected movement, trade and protection of germplasm. In addition, bananas produced using the tissue culture techniques are stated to be more energetic, higher yielding and make better quality fruits than those gives by conventional way (Hwan, 1976).

The crop is vegetative propagated by the help of different types of sucker's. However, this is time consuming method and gives the inadequate number of planting material. Methods of plant tissue culture and plant cell have helped in fast banana varieties multiplication and employing floral apices or tips of shoot (Cronuer and Krikorian 1986). For rapid propagation of clone meristem culture offers an efficient method production of materials free from virus and germplasm preservation in plants (Hwang Shinchuan et al., 2000; Helliot et al., 2002). Banana through the methods of *in vitro* propagation has been stated by a number of workers by different techniques and sources of explants (Jalil et al., 2003; Wong et al., 2006; Shirani et al., 2007). Since the germination frequency of seed is very low, embryo culture is favored for classical breeding experiments. Shoot tip culture major applications are mass clonal propagation and germplasm conservation. In mass clonal propagation existing shoot tips are stimulated to rapidly multiply while as in germplasm conservation multiplication rate is slowed down (Vuylsteke, 1993).

Mass propagation of preferred genotypes, some clonal variation methods, genetic engineering and other biotechnological approaches can be used for the development of banana crop which is established on consistent protocols of plant renewal. TC also plays a significant function in germplasm protection, distribution, and safe interchange of internal material for planting and newly chosen quick propagation of hybrid cultivars. A number of scientists have stated the rejuvenation of Musa spp. through micro propagation (Cronauer & Krikorian, 1986; Jarret, 1986; Diniz et al., 1999; Nauyen and Kozai, 2001; Krishnamoorthy et al., 2001; Kagera et al., 2004; Muhammad et al., 2004; Roels et al., 2005; Madhulatha et al., 2004). Under *in vitro* condition shoots growth and multiplication are affected by a lot of factors, one of which factor is the adding of source of carbon to the medium (Ill wan and Korban, 1998). Carbon sources gives as osmotic and energy agents to support the growth of the plant tissues (Lipavska and Konradova, 2004). Under *in vtro* conditions, on the growth of plants there are different ideas on the useful result of different carbon sources (glucose, fructose and sucrose). In the tissue culture, 2 to 5% sucrose is the mainly accepted carbohydrate used (Bridgen, 1994). *Linum usitatissium*, at 4% concentrations showed that medium supplemented with monosaccharide's glucose or fructose (Cunha and Fernandes-Ferreira, 1999). Though sucrose has been the carbohydrate in the vast majority of work on i*n vitro* shoot induction and shoots development in woody species, it is not always the most efficient carbon source for these purposes. Thus, the carbohydrate requirements have yet to be defined and optimized in micro propagation system (Cuenca & Vieitez, 2000). Excised tissues explants demand a constant energy supply to support the growth, allied physiological actions, multiplication and differentiation, (Gurel and Gulsen, 1998).

In common, the majority of the studies concerning tissue culture are performed with sucrose as the only source of carbon across the plasma membrane due to its efficient uptake. Under *in vitro* plant growth conditions, glucose also has stated the diverse effects. This study suggests a rapid banana *Musa* (spp.) basrai multiplication protocol using different concentrations of sucrose medium, dextrose and sorbitol. These study findings might be useful for micro propagation establishment of banana methods to produce rapid clones under *in vitro* growth condition.

II. MATERIALS AND METHODS

Experiment was assessed at the TC laboratory of Plant Pathology Section, Sindh Agriculture Research Insitute, Tandojam, Pakistan. Randomized Block Design (RBD) was used for this study to investigate the carbon sources, their concentrations for evaluate and optimize for banana basrai *Musa* (spp.) protocol under *in vitro* micro propagation condition.

Suckers were collected from Thatta district. The extra tissues were detached by trimming away the leaf basis corm tissues and outer leaf sheaths until a 5 to 7 cm cube enclosing the shoot apex obtained. The cubes of tissues were then washed for about 1 hour under tap water running. Under laminar air flow cabinet, the cubes were then disinfected for thirty min by soaking in viable bleach (5.25 percent NaOCl) and diluted to thirty percent (v/v) and with 2 drops of Tween 20 per 100ml and followed by

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rinsing with distilled water for three times in autoclave (Ganapathi et al., 1992).

Explants were then placed on sterile Petri dishes till the explants size reached about 1.5 to 2 cm of length, all brown tissues from the surface and outer leaves were detached and then explants cultured in the propagation media (Ganapathi et al., 1992). Consisted of full MS basal Salts effectiveness and full MS vitamins mixture with five milligram per litter BAP, 0.2 milligram per liter NAA, seven gram per litter agar and additional Phosphate (KH2Po4 17 g/l), the pH was used to 5.7 ± 0.1 with NoaH and HCL prior to adding agar (Murashige & Skoog, 1962). By autoclaving at 121°C and under a pressure of 15 psi, all the media and dishes were sterilized for 20 min (Ganapathi et al., 1992). In pure ethanol, forceps and the dissecting blades were dipped and exposed to gas flame and with help of 70% ethanol, laminar air-flow chamber was cleaned by spraying and wiping. Before 15 min of use laminar air-flow was switched on. During the night, ultra violet lamp was switched on in culture room.

Depending on experiment objective, either in dark or light chamber, the culture was maintained less than 16 hours light exposure of 1000 lux. The temperature of culture room was maintained at 25 ± 2 degree Celsius using white cool fluorescent lamps. We used basrai explants for the propagation of culture. Different concentrations of Sucrose, dextrose and sorbitol sugar were added to media i.e. 0 (control), 15, 30, 45 and 60g/l.

III. RESULTS AND DISCUSSION

In this study, influence of sucrose, dextrose and sorbitol sugars as carbon source and their varied concentrations were evaluate after six weeks of culture on the basal medium of MS.

3.1. Effect of sucrose sugar as carbon source and their various concentrations:

Results stated that 30g/l sucrose give maximum growth in all parameters respectively. However, 30gram per litter value of sucrose was considerably higher than the other values followed by 15g/l, control recorded low value (Table 1 and figure 1). 30g/l sucrose produces best results in all parameters; number of shoots (per explants), shoot length (cm), number of roots (per explants) and root length (cm). Our findings are conformity with other experts (Helliot et al., 2002) who stated that concentrations of sucrose at 30gram per litter gave significantly maximum shoots mean number in phase of Patchouli banana multiplication. (Jalil et al., 2003) who reported that the concentrations of sucrose (30 and 40g/l) advantage for clonal propagation of *Musa* (spp.) resulting in maximum growth parameters and number of suckers evaluated. (Noreldaim Hussein, 2012) stated that sucrose 3.0% was mainly optimum as expressed by better growth vigor at both systems of root and shoots tip culture of banana. (Buah et al., 2000) reported that sucrose at 30 g/l demonstrated the highest number of leaf RGR in sago. (Ekhlas Morfeine, 2014) reported in banana (*Musa spp*. cv Shima) the highest shoots number was acquire by 30gram of sucrose/l. This value was considerably greater than other values but 15gram of sucrose/l and 45gram of sucrose/l were not significant. (Madhulatha et al., 2006; Hussein, 2012) stated that sucrose has been the most usually used source of carbon for a great number of species of plants containing banana. However, (Li and Wolyn 1997) studied that addition of sucrose in the media stimulated both shoots as well as growth of root. These authors noted that a specific sources of carbon concentrations that carried highest multiplication. They emphasized the need of using different concentrations of sugar for improving the achievement of micro propagation.

3.2. Effect of dextrose sugar as carbon source and their various concentrations:

30g/l dextrose gave best results than other values followed by 15g/l; low value was again recorded by control (Table 2 and figure 2). Our findings are agree with (Ekhlas, 2014) who stated that both 45 gram per liter and 60 gram per liter of glucose and dextrose level energizing multiplication and were different source of carbon energy for propagation of *Musa* (spp.). **(**El-mana, 1999) also found the helpful reactions of dextrose or glucose as source of sole carbon energy in the cultures of strawberry.

3.2. Effect of sorbitol sugar as carbon source and their various concentrations:

Since highest growth was recorded on 45g/l of sorbitol sugar concentration as carbon source followed by 30g/l. Control recorded 0 values in all parameters (Table 3 and figure 3**).** Carbohydrates like sorbitol, glucose, galactose and maltose) may show greater to sucrose and can also be used in particular situation. Quantitatively, pervious results declare the decrease of the optimum sucrose to 3.0% level of concentration (Novak et al., 1986; Mateille and Foncelle, 1988). Sugar help as a major transfer of metabolites and is responsible for the nutrient uptake through the osmotic processes from the medium (Lalonde et al., 1999)**.** Vasil et al., 1982 stated that tissue culture method involves the establishment of different tissue or cell under an appropriate culture condition, following regeneration of plants and *in vitro* proliferation of cell. Collapse in most tissues or plant cell to get complete plants under *in vitro* circumstances is due to lack of appropriate method and inadequate information about

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nutrient media and other physical and chemical circumstances, which are necessary for proper development of cells, tissues and organs (Johri, 1982). The difficulty of rising diseases can be managed by propagating banana propagation through TC (Ali et al., 2011). Sugar supplies in any of the procedure of TC, in conditions of quantity, kind and differ in species, variety and plant growth stage (Singh and Shymal, 2001; Chun et al., 2008; Gurel and Gulsen, 1998). (Buah et al., 2000) recommended that fructose is not appropriate for in vitro culture in several banana varieties. In 'Shima' variety of banana, glucose and sucrose were established to be evenly appropriate. Fructose, on the other hand, was found to give the poorest results for all studied parameters. The basic aspects of carbon consumption, cell metabolism and TC that was not understood previously (Romano et al., 1995).

Authors distinguished that a precise carbon sources concentrations that carried highest growth. They emphasized the need of using unusual concentrations of sugar for improving the achievement of micro propagation.

4. FIGURES AND TABLES

4.1. **Figures**

Fig.1: Effect of different concentrations of sucrose sugar on micro propagation of banana Musa (spp.) Basrai explants after 6 weeks of incubation.

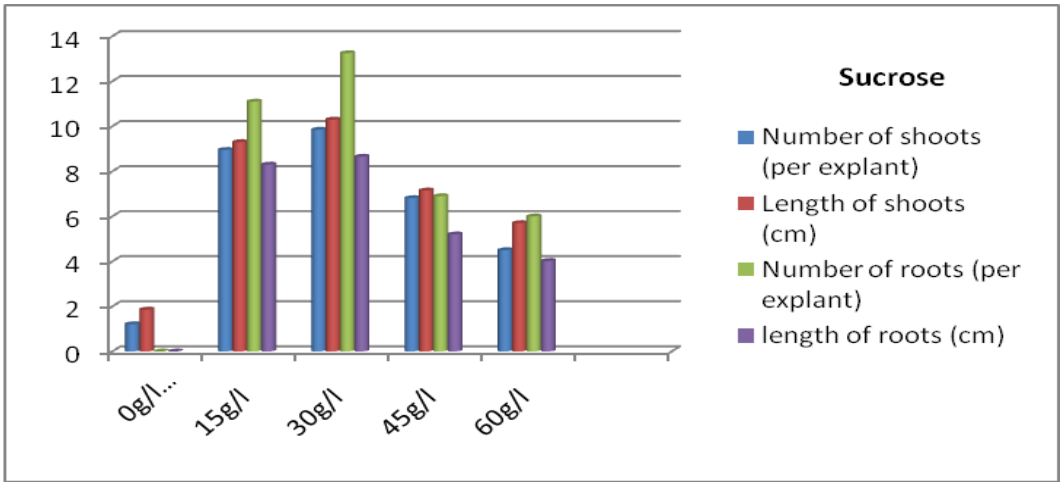

Fig.2: Effect of different concentrations of dextrose sugar on micro propagation of banana Musa (spp.) Basrai explants after 6 weeks incubation.

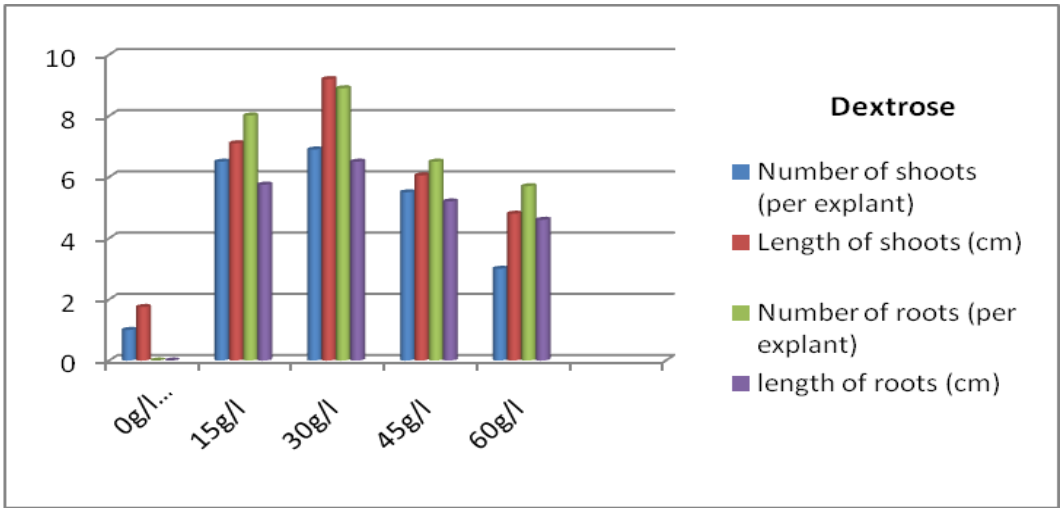

Fig.3: Effect of different concentrations of Sorbitol sugar on micro propagation of banana (Musa spp.) explants after 6 weeks incubation.

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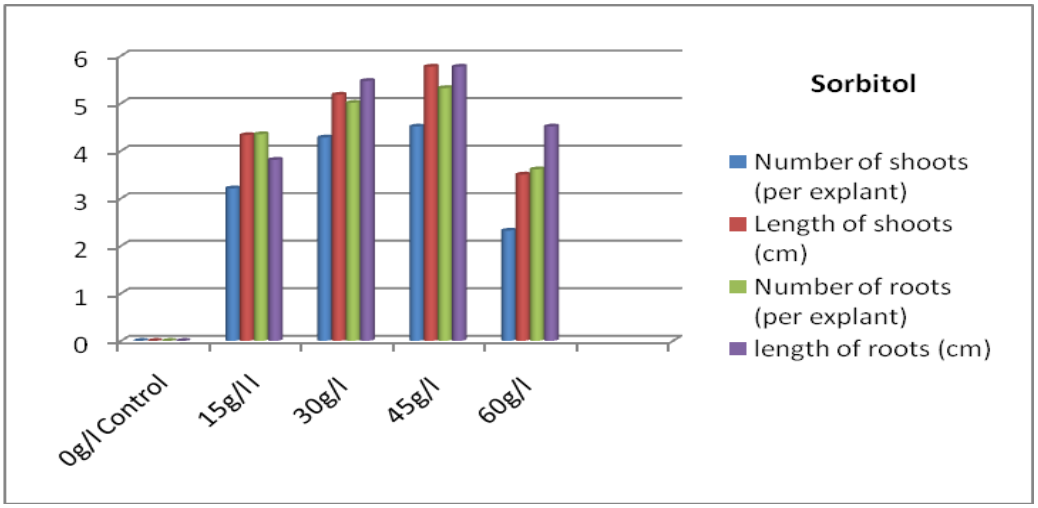

Table. 1: Influence of sucrose sugar concentrations on banana shoot explants growth under in vitro condition after 6 week of incubation.

4.2. **Tables**

Sucrose Concentration (g/l)	**Number of shoots (per explants)**	**length of shoot (cm)**	**Number of roots (per explants)**	**Length of root (cm)**
0	1.21 E	1.86 E	0.00 E	0.00 E
15	8.95 B	9.30 B	11.1 B	8.30 B
30	9.85 A	10.30 A	13.25 A	8.65 A
45	6.81 C	7.15 C	6.9 C	5.20 C
60	4.50 D	5.70 D	6.00 D	4.020 D
LSD	0.0608	0.0360	0.0346	0.0306
CV	0.1355	0.0801	0.0772	0.0681

Means within the same column followed by same letters are significantly different

Table. 2: Influence of dextrose sugar concentrations on banana shoot explants growth under in vitro condition after 6 week of incubation.

dextrose concentration (g/l)	**Number of shoots (per explants)**	**length of shoot (cm)**	**Number of roots (per explants)**	**Length of root (cm)**
0	1.00 E	1.75 E	0.00 E	0.00 E
15	6.50 B	7.10 B	8.01 B	5.75 B
30	6.90 A	9.20 A	8.90 A	6.50 A
45	5.50 C	6.05 C	6.50 C	5.20 C
60	3.00 D	4.80 D	5.70 D	4.60 D
LSD	0.0320	0.0253	0.0213	0.0207
CV	0.0714	0.0564	0.0474	0.0460

Means within the same column followed by same letters are significantly different

Table. 3: Influence of sorbitol sugar concentrations on banana shoot explants growth under in vitro condition after 6 week of incubation.

Sorbitol concentration (g/l)	Number of shoots (per explants)	length of shoot (cm)	Number of roots (per explants)	Length of root (cm)
0	0.00 E	0.00 E	0.00 E	0.00 E

15	3.21 C	4.33 C	4.35 C	3.81 D
30	4.28 B	5.18 B	5.01 B	5.47 B
45	4.51 A	5.77 A	5.32 A	5.77 A
60	2.32 D	3.50 D	3.61 D	4.51 C
LSD	8.944	0.0114	0.0126	0.0112
CV	0.0199	0.0253	0.282	0.0249

Means within the same column followed by same letters are significantly different

IV. CONCLUSION

We established suitable media with composition of different concentrations of sucrose, dextrose and sorbitol as a carbon source of banana basrai *Musa* (spp.) for micro propagation under *in vitro* circumstances. Demonstrated that higher growth vigor of sucrose 30g/l in all parameters as compared to dextrose, sorbitol and their variance concentrations. Sucrose 30g/l most optimum for banana tissue culture of *Musa* (spp.) basrai multiplication. Banana micro propagation through TC is consistent solution to the farmers that farmers facing. So, *in vitro* propagation techniques are efficient to overcome these challenges.

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